AssayMax™

Human HSP47 ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of Biotinylated Antibody per well.
Incubate 2 hours.

Step 3. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 4. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 25 minutes.

Step 5. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Human Heat Shock Protein 47 (HSP47) ELISA Kit

Catalog No. EH5202-1

Sample insert for reference use only

Introduction

Heat shock protein of 47 kDa (HSP47), also called serpin H1, clade H, member 1, collagen binding protein 1 and collagen, is a member of the serpin superfamily of serine proteinase inhibitors. HSP47 is a 418 amino acids collagen-specific molecular chaperone involved in the collagen folding and secretion. It localizes to the endoplasmic reticulum lumen and its expression is induced by heat shock (1, 2).

Principle of the Assay

The AssayMax Human Heat Shock Protein 47 (HSP47) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human HSP47 in **plasma, serum, milk, tissue extract, and cell culture lysates**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human HSP47 in less than 5 hours. A polyclonal antibody specific for human HSP47 has been pre-coated onto a 96-well microplate with removable strips. HSP47 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for HSP47, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human HSP47 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human HSP47.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human HSP47 Standard:** Human HSP47 in a buffered protein base (20 ng, lyophilized, 2 vials).
- **Biotinylated Human HSP47 Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against HSP47 (60 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrated (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes, use supernatants, and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Collect the serum and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Lysates:** Place the cell culture dish in ice and wash the cells with ice-cold PBS. Drain the PBS, then add ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Triton, 0.1 mM PMSF, 1 µg/ml leupeptin, 1 µg/mL aprotinin, and 1 µg/mL pepstatin). Scrape adherent cells off the dish and then transfer the cell suspension into a pre-cooled microfuge tube. Maintain constant agitation for 30 minutes at 4°C. Centrifuge in a microcentrifuge at 4°C. Collect fresh cell lysates. Use undiluted samples or dilute samples 1:2 with EIA Diluent and assay. The undiluted samples can be stored at -20°C or below.
- **Tissue:** Extract tissue samples with 50 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x *g* for 20 minutes. Collect the supernatant and measure the protein concentration. Use undiluted samples or dilute samples 1:2 with EIA Diluent and assay. The undiluted samples can be stored at -20°C or below.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 20 ng of Human HSP47 Standard with 0.4 ml of EIA Diluent to generate a 50 ng/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (50 ng/ml) 1:2 with EIA Diluent to produce 25, 12.5, 6.25, 3.125, and 1.563 ng/ml solutions. EIA

Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 48 hours.

Standard Point	Dilution	[HSP47] (ng/ml)
P1	1 part Standard (50 ng/ml)	50.00
P2	1 part P1 + 1 part EIA Diluent	25.00
P3	1 part P2 + 1 part EIA Diluent	12.50
P4	1 part P3 + 1 part EIA Diluent	6.250
P5	1 part P4 + 1 part EIA Diluent	3.125
P6	1 part P5 + 1 part EIA Diluent	1.563
P7	EIA Diluent	0.000

- **Biotinylated Human HSP47 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human HSP47 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Biotinylated Human HSP47 Antibody to each well and incubate for 2 hours.
- Wash the microplate as described above.

- Add 50 μl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 25 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

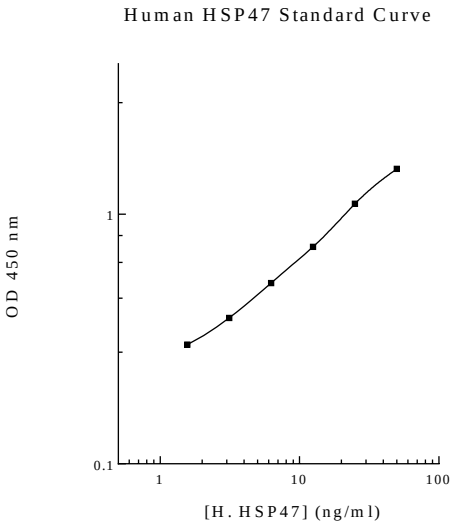
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	50.00	1.516 1.524	1.520
P2	25.00	1.103 1.101	1.102
P3	12.50	0.743 0.739	0.741
P4	6.250	0.533 0.532	0.533
P5	3.125	0.382 0.386	0.384
P6	1.563	0.300 0.300	0.300
P7	0.000	0.210 0.216	0.213
Sample: **Sodium Citrate Plasma (1x)**		0.378 0.380	0.379

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- Human plasma and serum samples from adults under mild amounts of daily stress and otherwise healthy were tested (n=20). On average, HSP47 level was 3.1 ng/ml.

Performance Characteristics

- The minimum detectable dose of HSP47 as calculated by 2SD from the mean of a zero standard was established to be 0.9 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	2.9%	3.0%	2.7%	7.4%	7.6%	7.9%
Average CV (%)	2.9%			7.6%		

Recovery

Standard Added Value	3 – 25 ng/ml
Recovery %	84 – 111%
Average Recovery %	96%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
No Dilution	95%	93%
1:2	105%	102%
1:4	111%	111%

Cross-Reactivity

Species	Cross Reactivity (%)
Beagle	None
Bovine	None
Monkey	<10%
Mouse	<10%
Rat	None
Swine	<50%
Rabbit	None

References

(1) Strausberg RL *et al.* (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99:16899-16903
(2) Razzaque MS *et al.* (2005) *Contrib. Nephrol.* 148:57-69

Version 1.6R2

Related Products

- EH5001-1 AssayMax Human HSP27 ELISA Kit (Plasma, Serum, Milk, Tissue Extract, and Cell Culture samples)
- EH5505-1 AssayMax Human HSP60 ELISA Kit (Plasma, Serum, and Cell Culture samples)